Poster

[P25-6] P25-6: Immunosuppressive drugs (1): LC-MS/MS assay

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[P25-6-5] Fully automated platform for sensitive determination of immunosuppressant drugs in whole blood using high quality internal standardization

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Background

Measurement of immunosuppressant drugs is essential during organ transplantation. Under-dosing can lead to organ rejection, while over-dosing can cause serious toxicity. Traditional methods to measure immunosuppressant drugs in whole blood are based on either immunoassays or chromatography. Immunoassays, though, are affected by matrix interferences and lack of specificity. LCMSMS has then become the gold standard due to its specificity, precision and sensitivity. However, it has still one major drawback: current LCMSMS platforms demand personnel with expertise and, for whole blood samples, tedious sample preparation. As a consequence, sample throughput is generally much lower than for immunoassays.

We here report a fully automated procedure for the quantitation of four major immunosuppressant in whole blood samples, using of 13C labelled internal standards.

Methods

The quantitative analysis of Immunosuppressant was performed using reagents provided in Alsachim Dosimmune kit. The Immunosuppressant and Internal standard were monitored using UHPLC-MS/MS system (Nexera X2 and LCMS-8050, Shimadzu, Kyoto). Sample preparation was performed using extraction buffer and internal standard set provided in Alsachim Dosimmune kit. Analytical performance of the method was monitored using whole blood calibrators and whole blood QC. Automatic sample preparation was performed using CLAM-2000 module (Shimadzu, Kyoto).

Results

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The method enables the quantification of tacrolimus, sirolimus, everolimus and cyclosporine-A in whole blood samples. The established quantification strategy for this compounds is to use internal calibration using deuterium labeled standards. However, they generally suffer from poor isotopic enrichment, leading to overestimation of the unlabeled form. We here use 13C labeled internal standards for tacrolimus, sirolimus and everolimus. This guaranties better isotopic enrichment, better precision of the results, long term stability of the standards and perfect co-elution with the analytes, leading to better corrections of matrix effects. Linearity was confirmed in the range 0.5-40 ng/mL for tacrolimus, sirolimus, everolimus, and in the range 5-1500 ng/mL for cyclosporine-A. For all analytes, r^2 of linearity models was above 0.99, with S/N >25 for LLOQ levels. Controls showed accuracies comprised in between 85 and 115% for all analytes.

Conclusions

Fully automated quantitation of immunosuppressant in whole blood, using of 13C labelled internal standards, increasing data quality, throughput and safety.